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(54) **Natural source composition for the control of post-harvest pathologies and method of application**

(57) The composition contains a fundamental active ingredient consisting of the association of thymol, eugenol and cinnamaldehyde, a secondary active ingredient consisting of the association of cuminaldehyde, geraniol, vanillin, borneol, menthol, anethole, terpineol, limonene and different glycosides contained in the essences of mustard and jasmine, a surface-active selected among the different families accepted as emulsifying

food additives and an oligosaccharide selected among the mano-oligosaccharides, fructo-oligosaccharides, phyco-oligosaccharides proceeding from algae extracts or vegetable tissue.

The composition that has just been described is applied to fruit and vegetables in a diluted bath or in solid form incorporated into the waxes that are usually used in post-harvest treatments.

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Description

[0001] The present invention consists of a composition for the control of post-harvest pathologies of fruits and vegetables, specifically the attack of pathogens and the phenomenons of chemical degeneration of the skin, with the particularity that its application is compatible with human or animal consumption.

[0002] Within the process of preservation of fruits and vegetables the two most important problems that arise are:

- The attack of pathogenic agents, mainly specific types of funguses and bacterias.
- The process of chemical degeneration of the skin, which is defined in two fundamental phenomenons: scalding of the skin -bruising- (mainly apples and pears), which seems to be linked with determined oxidation processes (the generation of highly reactive free radicals), and oleocellosis (citrics).

[0003] Actually, both processes -included among what we may call post-harvest pathology- are partly related in spite of the differences that exist in the mechanisms that generate them.

[0004] In effect, it is known that the processes of pathogenic attack generates a local response in the vegetable tissue related to the natural defense mechanism, which includes the synthesis of highly reactive oxygenated species (H_2O_2 , $*O_2$ and $*OH$) which may produce cellular death by means of self-oxidation processes. In this context, it is very probable that the oxidation of substances in the area of the skin may be produced -such as a-farnesene in the case of scalding- which will aid the scalding processes, as well as the processes of oleocellosis as a response to the generation of toxic substances (Bondoux, P., "Diseases in the preservation of fruits and vegetables." INRA, Mundi Prensa, 1994; Snowdon, A., "Post-harvest. Diseases & Disorders of fruits and vegetables." Wolfe Scientific, 1990; Tuset, J.J., "Citric rotting," Generalitat Valenciana, 1987.)

[0005] For this reason we consider it fundamental to address the solving of post-harvest pathology in an integrated way, so that the two general problems may be handled simultaneously and coordinately: the affliction of pathogens and the chemical degeneration of the skin.

[0006] Presently synthesis products have been developed that are efficient against both problems - thiobendazols, benomyl or orthophenyphenol for the control of funguses and other pathogens; and phenylamine or ethoxyquin for the control of scalding-which however present many toxicity problems for human health, both related to the consumption of the treated products as well as in their application to the fruit or vegetable. Likewise, none of them simultaneously and coordinately solves the two problems included in post-harvest pathology.

[0007] Due to all of this, the development of eco-compatible products, without any type of:

- Phytotoxicity or decreasing effect in the quality -taste, smell, color, texture- at efficient doses.
- Risk for human health, both in immediate consumption of the treated product as well as in the process of application of the product.

[0008] With the capacity of controlling coordinately and in a simultaneous way the problems of pathogenic agent attacks and the problems of chemical degeneration of the skin -scalding and oleocellosis- they undoubtedly are of interest and they constitute a presently unresolved aspect. As we will see next, this is the object of the present invention.

[0009] Presently, non-toxic natural source compositions have been developed -derived fundamentally from essential oils of vegetable source accepted for the direct use in human nutrition as an additive- with the capacity of controlling the attack of funguses in producing plants, however these compositions are not valid for the overall control of post-harvest pathology for two fundamental reasons:

- The described doses and their method of application reveal phytotoxicity in citrics due to the development of an oleocellosis process that generates bruising (processes of oxidation-generation of highly reactive free radicals). In fact, the majority of these patents describe the use of the product in producing plants either in foliar application or in radicular application, but not in post-harvest application.
- They do not have the capacity of controlling the processes of chemical degeneration of the skin. None of these patents describes an action of the product against the problems of scalding or oleocellosis.

[0010] Thus, the French patent FR 2529755 (Sotome, 1982) describes the use of cinnamic aldehyde (cinnamaldehyde), a major component of cinnamon essence, as an agent for the control of pathogenic funguses both in producing plants -foliar or radicular use- as well as in the post-harvest processes. However, as shown in table 1, the direct application of the product according to the composition and dose described by the author, possesses a high level of phytotoxicity in citrics, by unleashing an oleocellosis process in the skin.

Table 1.

<p>Intensity and frequency of the phytotoxicity derived from the application of cinnamic aldehyde (cinnamaldehyde) and the association of cinnamic aldehyde-eugenol in oranges (Valencia late). (Time of application: 60 seconds. No. of fruits: three repetitions of three fruits. Temperature 20° C. Method: immersion bath.)</p> <p>Frequency % of affected fruit.</p> <p>Intensity % of surface of affected skin.</p>		
Treatment	Frequency	Intensity
- 20% Cinnamaldehyde	100	90
- 15% Cinnamaldehyde, 15% Eugenol	100	90

[0011] On the other hand, the described composition does not have any effect on the control of the scalding or oleocellosis processes, and in this last case it even provokes it.

[0012] Maybe these results led this same author to a later patent with a similar content. The document of American patent US 4,978,686 (Sotome, 1987) describes a composition consisting of the association of a cinnamaldehyde and an antioxidant, but only for use in growing plants -foliar or radicular application- and **not for post-harvest use**. Likewise, it does not describe any anti-scalding use or effect or the control of oleocellosis in citrics. In effect, the introduction of the antioxidant is only to inhibit the oxidation of the cinnamic aldehyde in the means. The introduction of the antioxidant is not directed toward an action on the plant.

[0013] On the other hand, the inclusion of a surface-active is aimed only at facilitating the dispersion of the product in water. In fact, the products advised by this author are not admitted as additives for use in food (Pluronic L-64 and Pluronic P-102).

[0014] Beilfuss et al, in the Canadian patent document CA 2,012,288 describe a composition consisting of a phenol (including thymol, eugenol, carvacrol), an alcohol (e.g. of the phenoxy-ethanol group) and a surface-active (not described). With a disinfectant effect on the plant (foliar and ground application) and of surfaces and rooms. He does not describe its use on fruit and vegetables in post-harvest application. Likewise, he does not describe any anti-scalding effect or of oleocellosis control in citrics.

[0015] Other patent documents are known which contain some essential oil, but their application is focused on an insecticide, nematicide, fungicide or bactericide action, always on producing plants (foliar or ground application), but not for post-harvest use. Likewise they do not describe any anti-scaldant effect or of oleocellosis control in citrics.

[0016] Specifically, and as indicated previously, the object of the present invention is to obtain a composition which, when applied as described, allows:

- The application of a non-toxic, eco-compatible product and valid related with human nutrition, of **specific post-harvest use** for the control of the disorders associated with this situation: post-harvest pathology.
- To **control simultaneously and in a coordinated manner** the combination of fundamental problems associated with the denominated post-harvest pathology; the affliction by pathogenic agents and the phenomenons of chemical degeneration of the skin (scalding, oleocellosis) .

[0017] In summary, the object of the present invention is a new composition that:

- is eco-compatible
- does not have any risk for human health related both to the consumption of the products treated with it, as well as with the user in the application process.

[0018] With the capacity of simultaneously controlling the fundamental post-harvest disorders (post-harvest pathology):

- Affliction of pathogenic agents: mainly funguses, insects, bacterias and nematodes.
- Processes of chemical degeneration of the skin: scalding and oleocellosis.

[0019] Consisting mainly of:

- The association thymol-eugenol or cinnamaldehyde-eugenol or thymol-cinnamaldehyde-eugenol (or of the essences that contain them such as Thyme (thymol), clover (eugenol), and cinnamon (cinnamaldehyde); or analo-

gous products, structural of each one of them (these main associations can also be associated with the following secondary active ingredients: cuminaldehyde and/or geraniol and/or vanillin and/or borneol and/or menthol and/or anethole and/or terpineol and/or limonene and/or different glycosides contained in the essences of mustard or jasmine; or/and to the vegetable essences that contain them.

- 5 - A surface-active among the different families accepted as emulsifying food additives such as lecithin, ammonium phosphatides, fatty acid esters, polysorbates, sucrose esters and saccharose esters and fatty acids and polyglycerides. Preferably sorbitans and polysorbates (Twens and Span families).
- Determined oligosaccharides: mano-oligosaccharides; fructo-oligosaccharides; phyco-oligosaccharides proceeding from algae extracts (e.g. ascophyllum nodosum) and phyto-oligosaccharides proceeding from any vegetable
- 10 tissue.
- And a method of application consisting in that these compositions are used by means of a bath (drencher, shower or immersion) of the product to be treated respecting the following parameters:
- Dose: between 1-10000 ppm in the application solution.
- Temperature: between 50-80° C.
- 15 - Time: between 1 second and 10 minutes.

[0020] The objects pursued by the composition object of this invention are reached by means of the following mechanisms:

20 Control of the affliction of pathogens:

[0021] Combined action of the main active ingredients thymol-eugenol; cinnamaldehyde-eugenol and thymol-eugenol-cinnamaldehyde (or structurally analogous to these) (we will call them EOI, meaning: Essential Oil Ingredient), formulated together or in the presence of other secondary active ingredients contained in other essential oils (see the summarized description of the invention) and determined oligosaccharides (OLS).

25 [0022] The EOI's would act by means of a direct pesticide action against the pathogen. The OLS's do not possess a direct action, rather an indirect one by means of the activation of the defense system of the plants.

[0023] The combined action of the two families of active ingredients allow to effectively inhibit the growth of the pathogenic agents, allowing a sufficiently reduced dosing of the EOI's so that the phenomenon of phytotoxicity described for other non-viable compositions (Beilfuss, Sotome) does not appear.

30 [0024] This strengthened action with phytotoxicity elimination, is favored by the method of application: a combination of the temperature of the bath and a reduced contact time of the fruit with the product.

[0025] In this case the surface-active agent is not fundamental, with its purpose being -on the other hand, important- the dispersion of the product in the aqueous means.

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Inhibition of the phenomenons of chemical degeneration of the skin.

[0026] This action takes place by means of two complementary mechanisms:

- 40 1. The elimination or reduction of the affliction caused by pathogenic agents limits the endogenous production of reactive oxygenated species (H_2O_2 , $*O_2$ and $*OH$), reducing the risks of oxidation of compounds present in the skin.
- 2. The combined presence of the specific thymol-eugenol-surface-active agent contributes toward creating a reducing atmosphere in the means, which allows the control of the authorization phenomenons.

45 [0027] In this second mechanism the combined presence of the three ingredients is found to be important to obtain a significant effect.

[0028] Specifically, the action of the selected surface-active agent is especially of interest, a type of Twen or Span (polysorbates or sorbitan esters; all of them valid for nutritional use); with Polysorbate 80 (Twen 80) proving to be especially efficient, and Span 80 at a concentration in the formula of 30% over the total composition. The presence of

50 the surface-active strengthens the control effect of the processes of chemical degeneration of the skin by improving the penetration and permanence of the active ingredients (thymol-eugenol).

[0029] For this action, the effect of the temperature or the time of exposure is not found to be so determinant.

[0030] In a preferred embodiment of the invention the method of operation is as follows:

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Preparation of 1 kg of product:**[0031]**

5 - Ingredients:

- 1. 300 g of thymol
- 2. 200 g of cinnamaldehyde
- 3. 120 g of eugenol
- 10 4. 50 g of cuminaldehyde
- 5. 30 g of mano-oligosaccharides
- 6. 300 g of Twen 80

15 - Manufacturing method

- 1. Introduce ingredients 1, 2, 3, 4 and 6 into a reactor with a blade mixer. Mix during 60 minutes at room temperature (20° C). It is not good for the temperature to be above 30° C to avoid the volatilization of the active ingredients 1, 2, 3, and 4.
- 2. Add compound 5. Mix the combination during 60 minutes.

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Application method**[0032]** In a bath, diluted

- 25 - Concentration: 200 to 1000 ppm
- Temperature: 45 to 50° C
- Time: 50 seconds

30 **[0033]** Alternatively the composition may be applied desiccated, in solid form, incorporated into the waxes that are used in post-harvest treatments.

[0034] Following, some field tests have been compiled. These tests were performed in real application conditions to contrast the efficiency of the composition and the method of application object of the present invention.

35 1. Disease control test:

[0035] The product used was of the following type: (Formula 1):

- Thymol: 20%
- Eugenol: 15%
- 40 Cinnamaldehyde: 15%
- Surface-active complex (Twen 80 type): 20%
- Mano-oligosaccharides: 5%
- Water: 25%

45 **[0036]** Its denomination is Formula 1**[0037]** Test conditions:**Varieties:** Pears, Abate and Conferencia**Method of treatment:** immersion50 **Duration of the treatment:** 60 seconds**Type of preservation:** refrigerator (Abate) and controlled atmosphere (Conferencia)**Period of drying before refrigerating:** 3 hours in open air.**Treatments:**

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N	Treatment	Composition	Dose (l/m ³)
1	Control	Drinking water	-

(continued)

N	Treatment	Composition	Dose (l/m ³)
2	Conventional	DPA* + TBZ*	2 + 1.2
3	Formula 1	Composition-invention	10

*DPA: Diphenylamine // TBZ: Thlobendazol

Sampling: at 108 days.

Results:

Abate Pear					
Treatm.	Total fruits	Healthy fruit	Pathogenic fruit	% pathogenic fruit	Scalded
1	96	91	5	5.2	0
2	96	93	3	3.1	0
3	96	96	0	0	0

Conferencia Pear							
Treatm.	Total fruits	Healthy fruit	Pathogenic fruit	% pathogenic fruit	Scalded	Fruit with phytotoxicity	% fruit with phytotoxicity
1	90	85	5	5.6	0	0	0
2	90	87	3	3.3	0	2	2.2
3	90	90	0	0	0	0	0

[0038] As can be seen, the formula object of this invention presented an efficiency equal to or -even-higher than the synthesis treatment of reference, which included thiabendazol as an antifungus agent and diphenylamine as an anti-scalding agent.

[0039] In these tests the anti-scalding effect could not be evaluated because the problem did not occur. However, in the conference pear, the phytotoxic effect can be observed of the conventional treatment which is not observed in our case.

2. Test of diseases and scalding control.

[0040] Products tested object of the invention.

[0041] Formula 1 (formula object of the invention indicated in the previous section.)

[0042] Formula 2 (formula object of the invention in which the antioxidant power of the compound thymol-eugenol-surface-active (Twen 80 type) is increased):

Thymol: 20%

Eugenol: 15%

Cinnamaldehyde: 15%

Surface-active complex (Twen 80 type): 35%

Mano-oligosaccharides: 5%

Water: 10%

Variety: Granny Smith apple

Method of treatment: immersion

Duration of the treatment: 60 seconds

Type of preservation: regular refrigerator (-0.3 to -0.5° C)

Period of drying before refrigerating: 3 hours in open air.

Treatments:

N	Treatment	Composition	Dose (l/m ³)
1	Witness	As collected	-
2	Conventional	DPA* + TBZ*	6 + 1.5
3	Formula 1	F. invention	10
4	Formula 2	F. invention	10

Sampling: at 115 days.
Results:

Treatment	Total fruits	Disease	Scalding
1	128	1	44
2	115	0	50
3	137	0	37
4	133	0	6
*DPA: Diphenylamine // TBZ: Thiobendazol			

[0043] Even though this type of apple did not present high levels of attack, the obtained results regarding the scalding are very interesting. As can be seen, formula 1 presented a significant reduction of the number of scalded fruits both with regard to the control as well as with the treatment with the synthesis product (diphenylamine).
[0044] On the other hand, it is observed that the strengthening of the system thymol-eugenol-surface-active complex significantly improves the capacity of controlling the scalding while maintaining the capacity of controlling the disease.

Claims

1. Natural source composition to control the post-harvest pathology of fruits and vegetables characterized for comprising:

1. A fundamental active ingredient, consisting of the association of thymol, eugenol and cinnamaldehyde or by the association of eugenol and one of the other two mentioned components.
2. A secondary active ingredient, consisting of the association of cuminaldehyde and/or geraniol and/or vanillin and/or borneol and/or menthol and/or anethole and/or terpineol and/or limonene and/or different glycosides contained in the essences of mustard or jasmine, the vegetable essences that contain the compounds that have just been mentioned.
3. A surface-active among the different families accepted as emulsifying food additives such as lecithin, ammonium phosphatides, fatty acid esters, polysorbates, sucrose esters and saccharose esters and fatty acids and polyglycerides.
4. An oligosaccharide constituted by a compound selected among the group of mano-oligosaccharides, fructo-oligosaccharides, phyco-oligosaccharides, proceeding from algae extracts (e.g. ascophyllum nodosum) and phyto-oligosaccharides (proceeding from vegetable tissues).

2. Natural source composition to control the post-harvest pathology of fruits and vegetables according to claim 1, characterized in that the mentioned surface-active has been selected among the sorbitan esters and polysorbates, that is, compounds from the Span and Twens families respectively.

3. Natural source composition to control the post-harvest pathology of fruits and vegetables according to claim 1, characterized in that its percentage composition by weight is:

1. Between 50 and 70 % of the main active ingredients
2. Between 5 and 20 % of the secondary active ingredients
3. Between 10 and 30 % of the surface-active selected among Twen 80 and/or Span 80
4. Between 1 and 5 % of oligosaccharides

4. Application method of a natural source composition to control the post-harvest pathology of fruits and vegetables, characterized in that it consists of a bath of the product to be treated in a solution of 1 to 10000 ppm concentration, at a temperature between 5 and 80° C and during a time comprised between 1 second and 10 minutes.
- 5 5. Application method of a natural source composition to control the post-harvest pathology of fruits and vegetables according to claim 4, characterized in that the concentration is of 200 to 1000 ppm, the temperature is maintained between 45 and 50° C and the time is 50 seconds.
- 10 6. Application method of a natural source composition to control the post-harvest pathology of fruits and vegetables, characterized in that the composition, in solid form, is added to waxes usually used in post-harvest treatments.

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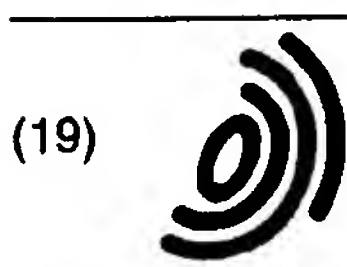
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EUROPEAN SEARCH REPORT

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Place of search THE HAGUE		Date of completion of the search 9 May 2001	Examiner Guyon, R
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			

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